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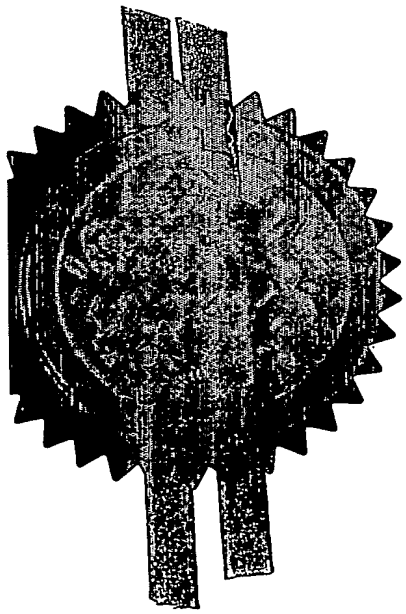

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Signed Andrew Gerry

Dated 21 July 2003

Patents Form 1/77

Patents Act 1977
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1/77

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13JUN02 E725325-1 [illegible]
P01/7700 0.00-0213481.5

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — IS/FP5967682

2. Patent application number
(The Patent Office will fill in this part) — 0213481.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
Patents ADP number *(if you know it)*

Medipearl Pte Limited
No. 1 Third Chin Bee Road
SINGAPORE 618679

8401044001

If the applicant is a corporate body, give the country/state of its incorporation — SINGAPORE

4. Title of the invention — PHARMACEUTICAL COMPOSITIONS

5. Name of your agent *(if you have one)* — MEWBURN ELLIS

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

YORK HOUSE
23 KINGSWAY
LONDON
WC2B 6HP

Patents ADP number *(if you know it)* — 109006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month /year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer "Yes" if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) — Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	11
Claim(s)	-
Abstract	-
Drawing(s)	4 + ff

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Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(Please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature

Date
11 June 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Ian Stuart

0117 926 6411

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PHARMACEUTICAL COMPOSITIONS

The present invention concerns compounds which are therapeutically active against some types of cancer. Thus it provides compounds, compositions, methods of manufacturing compositions and methods of treatment.

One of the innumerable plants used in Chinese traditional medicine is Fagopyrum dibotrys (or Fagopyrum cymosum meisen). The whole plant, particularly the rhizome, is used as a medicament, allegedly having a wide range of beneficial effects, including antitumour activity.

Zhang Wen-Jie et al., Acta Botanica Yunnanica, 1994, 16, 354-356 separated and identified a number of phenolic constituents. The compound obtained in highest yield (0.19%) was termed procyanidin B-2 and was assigned the formula (1) (see Fig 1). This compound has 5 asymmetric centres (asterisked in Fig 1), so potentially there are 32 stereoisomers. No information is available about which isomer(s) is/are present in the isolated material. They are 5,7,3′,4′- tetrahydroxy flavon-3-1 C_4 - C_8 dimers. Such a dimer or dimers was previously isolated from avocado seed (T.A. Geissmann et al. Phytochem., 1965, 4, 359-368).

We have now obtained the material from rhizomes of Fagopyrum dibotrys and have demonstrated remarkable and wholly unexpected levels of activity against a number of cancers. Clinical trials have employed a relatively crude extract of the plant material. Small amounts of purified compound have also been obtained, and tests on cell lines have supported the view that the procyanidin B-2 is the active ingredient.

Thus in various aspects the invention provides:

(a) the use of rhizomes of Fagopyrum dibotrys in the manufacture of a medicament for use in the treatment of cancer;

(b) the use of procyanidin B-2 as isolated from Fagopyrum dibotrys in the manufacture of a medicament for use in the treatment of cancer;

(c) the use of a compound of formula (1) in the manufacture of a medicament for use in the treatment of cancer;

(d) a process of producing a composition derived from rhizomes of Fagopyrum dibotrys suitable for use in cancer therapy;

(e) a method of cancer therapy comprising administration of a medicament which is a composition derived from rhyiomes of Fagopyrum dibotrys or procyanidin B-2 as isolated from Fagopyrum dibotrys.

Material can be obtained from plant material by extraction with a lower (C_1 - C_4) alcohol, preferably ethanol or methanol. This extract can be further purified by solvent extraction etc and by chromatography.

The material obtained from the plant or a compound isolated therefrom may be formulated in various ways for use in therapy. Conditions which may be treated include, for example, neoplastic diseases, particularly lung cancer and breast cancer. In accordance with this aspect of the present invention, the compounds provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors.

A compound may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, a pharmaceutically acceptable excipient,

carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such a gelatin.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Production of Extract

Samples of Fagopyrum dibotrys were obtained from parts of China: Yun Nan Province; Long Quan District of Sichuan Province, Liang Shan District of Sichuan Province, and Chong Qing County of Sichuan Province. The material from Liang Shan appeared to be of the best quality and analysis showed it had by far the highest content of ketones (7.65% by weight), and a significantly higher tannin content (2.90% by weight). Therefore this material was used for further study.

Fagopyrum dibotrys rhizomes were broken into small particles, and 2400kg of this particulate material was extracted with 70% ethanol 12 times using a total of 28,800kg. The extract was concentrated by evaporation to give 1896 litres, 2184 kg. Half of this concentrate was further concentrated by evaporation under reduced pressure, which gave 278kg of syrup. The other part was spray dried, which gave dry powder (80kg). The syrup could be converted into a dry solid by heating in an oven. The solid could then be powdered.

The powder (spray or oven dried) contained 4.63% water, 35.35% ketones and 46.62% tannins, all by weight.

Purification of Procyanidin B-2

Powder (2kg) obtain as described above was extracted with technical ethanol. After warming at 65°C for 2 hrs, the solution was filtered. 13.2g of mixture was obtained after removal of the solvent from the filtrate. The herb was macerated again overnight and warmed for 2 hrs at 65°C and filtered. 8.2g of brown residue was obtained from the second filtrate. TLC analysis of the two extractions showed that the constituents of them are essentially the same.

For the purification of Procyanidin B-2 with chromatography, we found that by using ETHYL ACETATE: ACETIC ACID: WATER = 450: 10: 10 as eluent, most of the non-polar components could be removed. Three belts in the column were observed during the washing, the first one is green; the second one is red and the third one is brown. After the brown belt was washed down, Procyanidin B-2 was detected in the fractions collected. The relatively pure fractions were found to be suitable to view the component on TLC board, but not pure enough to run an NMR spectrum.

The detailed purification of Procyanidin B-2 on a column was carried out as follows: 100g of the crude extract was dissolved in 1 L water with strong stirring. The dark solution was extracted twice with ethyl acetate

(2 x 500 ml). A brown glass (10.6g) was obtained after removal of the solvent under vacuum. This step of purification could possibly remove most of the salts from the extraction.

120g of silica gel was loaded into a column with hexane to reach the length of 60cm. The brown glass (10.6g) was dissolved in 15ml ethyl acetate and added into the column. The column was washed with ETHYL ACETATE: ACETIC ACID: WATER = 700: 10: 10 to get a mixture. In order to recover the silica gel, the column was washed with 500ml water, followed by 500ml methanol and then 500ml ethyl acetate. The mixture obtained from the first run was loaded into this column again and eluted by the same solvent system. However, the fractions collected are still not pure enough. The 3.6g mixture obtained was a pale yellow glass after being vacuum dried.

The mixture (3.6g) was purified again by repeating the above mentioned procedures to get 0.29g Procyanidin B-2. On TLC plate, its purity seems quite good. In its NMR spectrum, we could observe the resonances reported by Zhang et al. (op.cit.) but some impurities which cannot be identified are also present. Its Mass spectrum shows the molecular ion at m/z 577.2 ([M-H]) as the highest peak. Another peak at m/z 289.2 suggests that the

fragment is the ion generated by breaking the C4-C8″ bond of Procyanidin B-2.

An alternative method to purify Procyanidin B-2 is to elute the column with ethyl acetate/hexane as a gradient solvent system (increasing the volume ratio from 1/1 to 2.5/1) to remove most of the components that are less polar than Procyanidin B-2 (The washing is slow yet efficient). The mixture containing Procyanidin B-2 is collected and purified further with the first method.

The detailed spectroscopic data of Procyanidin B-2 are summarised as follow: UVλ^{MeOH} (1gε): 208(4.96), 281(3.95); FAB-MS m/z: 577$[M-H]^-$; ^{1}H-NMR [$(CD_3)_2CO$]; δ2.73(1H, br, J = 16.0Hz), 2.89(1H,dd J = 16.0, 4.0 Hz), 3.98 (1H, m), 4.32(1H, m), 4.71(1H, s), 4.98(1H, br), 5.05(1H, br), 5.93-6.03(3H, m), 6.64-6.96(6H, m).

Biological Activity

We have found that procyanidin B-2 from Fagopyrum dibotrys possesses significant anti-tumour activity. In this study, it is named "MPCB". It was found to inhibit the production of matrix metalloproteinases from tumour cells, particularly IV collagenases. We have found that the invasion of B16-BL6 melanoma cells through the basement membrane was inhibited by MPCB in a concentration-dependent manner. We also investigated the

therapeutic effects of multiple oral administration of MPCB on mice inoculated with B16-BL6 melanoma cells. The administration of MPCB also significantly reduced the metastasis incidence as compared to untreated controls.

In a phase I study, 11 patients aged 11 to 78 with advanced NSCLC who have failed all conventional chemotherapy were given MPCB in escalated doses. This was in the form of soft-shell capsules containing the plant extract (powder) described above. Capsules were administered orally. The highest dose achieved was 7.2 grams daily (18 capsules, administered in 3 doses at different times) and no significant side-effects were encountered at this dose. Two of the 9 patients had stabilisation of disease, with a median survival of 9.5 months, instead of the expected median survival of 4 to 5 months for the entire group. In addition, the CEA level showed signficant reductions in some of the patients. In fact, there was significant reduction in tumour mass on CT scan evaluation of one of the patient.

Fig 2 A and B show CT scans of the patient taken 3 months apart. (Ai and Bi show scans at higher levels than Aii and Bii.) X indicates a lung, Y indicates the heart and Z indicates the aorta. The cancerous growth (lung cancer) is the white area indicated by the arrow C.

It can be seen that it is much reduced in the second scan.

In an acute toxic test of the drug on mice, the calculated LD50 is 61g/kg; 95% confidence interval 48.73g/kg to 77.02g/kg. According to the standard physiology index ratio between mouse and human beings, the calculated LD50 is 183.78g/man(60kg); 95% confidence interval 146.19 to 213.06g/man. Based on this result, the dosage of 10g/day is only 5.4% of the LD dosage. In addition, it was demonstrated that at low doses, as used in this study, indices such as WBC, Platelet, RBC, RDW and MCHC did not display significant changes. A sample of the drug was submitted to the Health Sciences Authority for analysis and no significant toxic compounds such as heavy metals were detected. The method of extraction and processing of the herb has received certification from the Sichuan Health Authorities.

Figure 3 A-D shows the results of tests of the purified B-2 compound on human breast cancer cells grown in tissue culture. Figs 3 A and B show control cells at magnifications of 10 and 20 times. Figs 3 C and D are corresponding views of cells treated with the drug. The marked reduction in the number of cells is apparent.

Figures 4 A-D are similar to Figs 3 A and D but relate to human liver cancer cells. Figs 4 A and B show control cells at 10 and 20 times magnification whereas Figs 4C and 4D show treated cells. Once again it is evident that the number of cancer cells was substantially reduced by the drug.

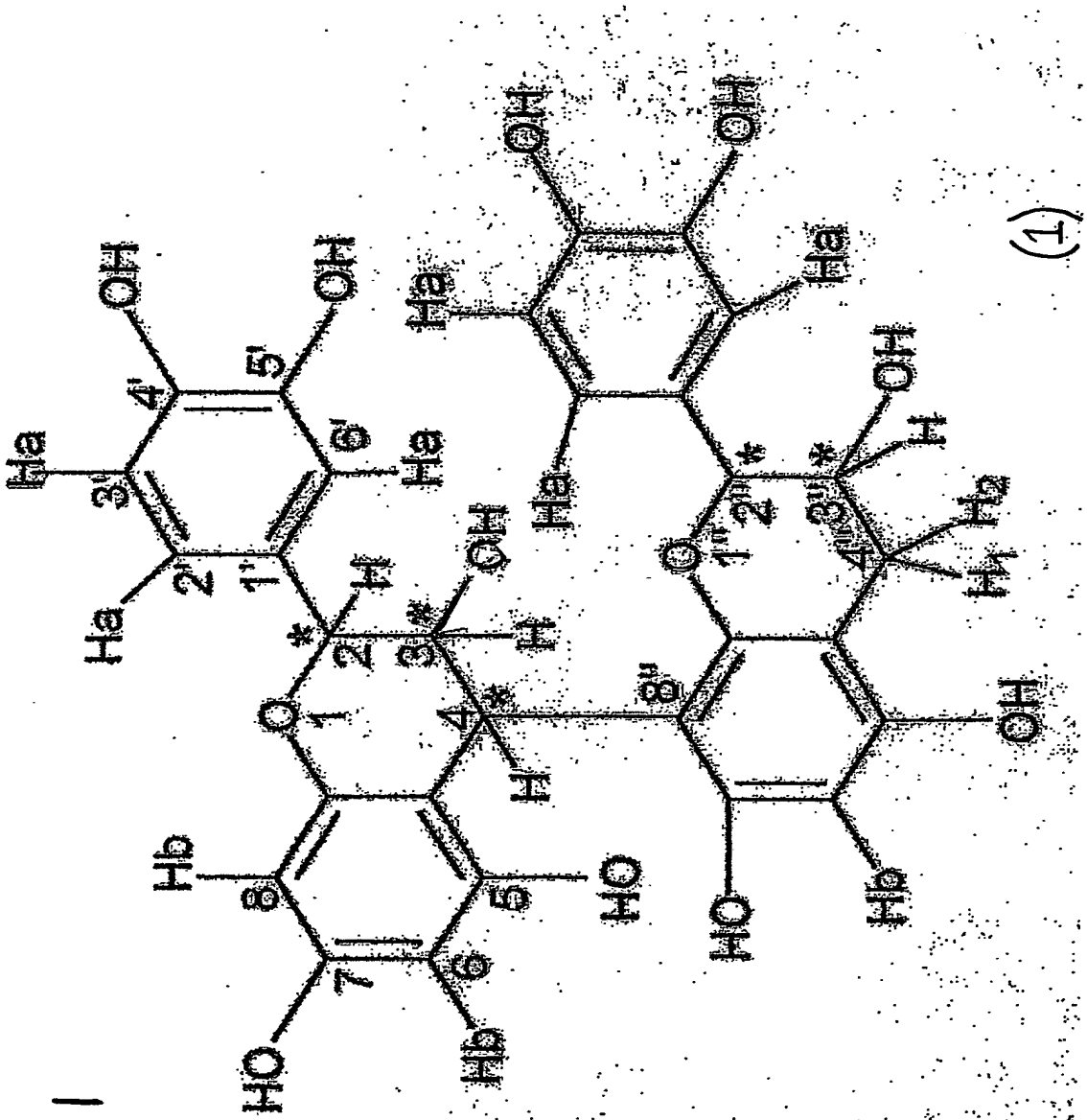

Fig 1

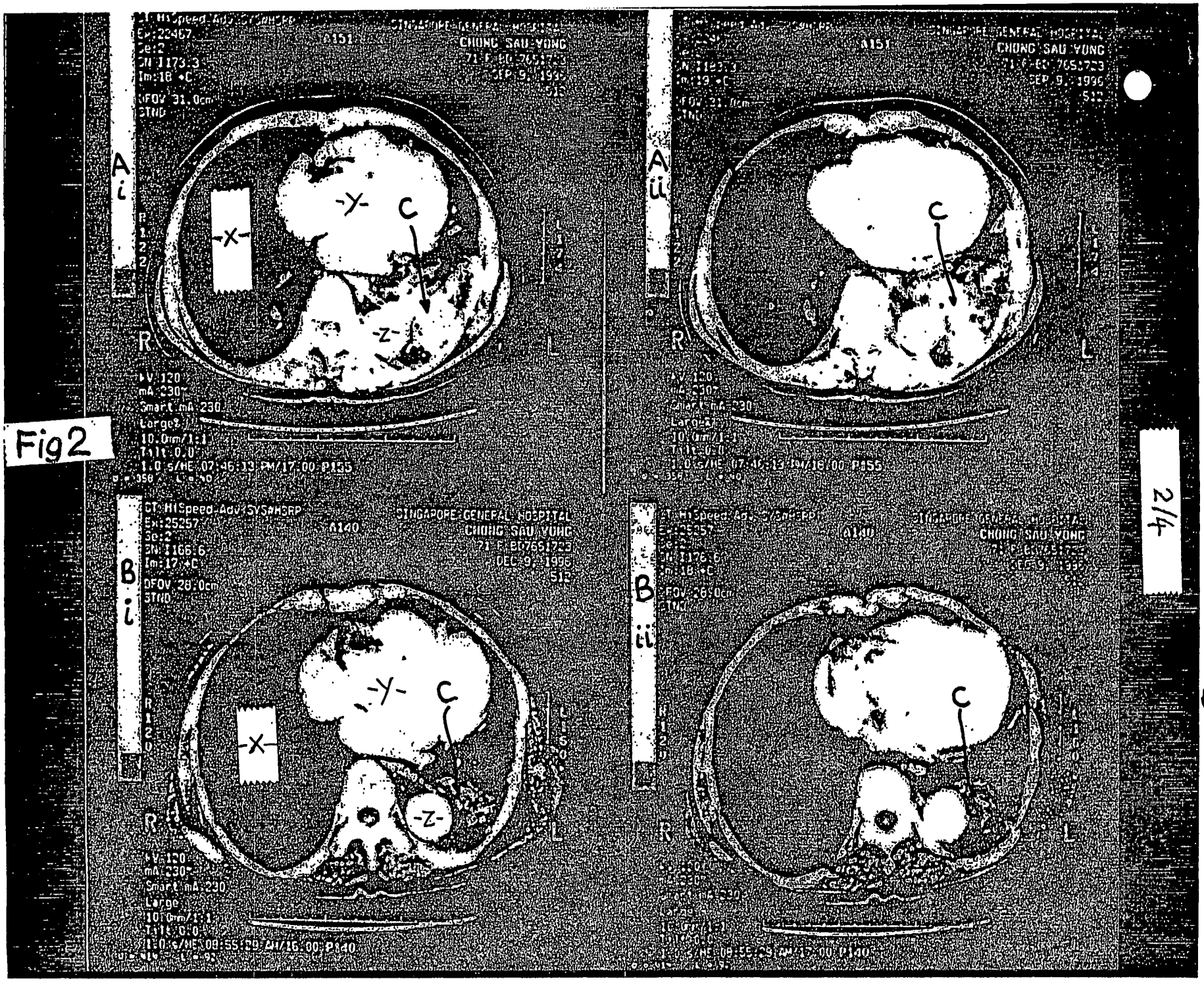
Ai
Aii
Bi
Bii
-X-
-Y-
-Z-
C
R
L
CHONG SAU YONG
SINGAPORE GENERAL HOSPITAL
SEP 9, 1996
DEC 9, 1996

Fig2

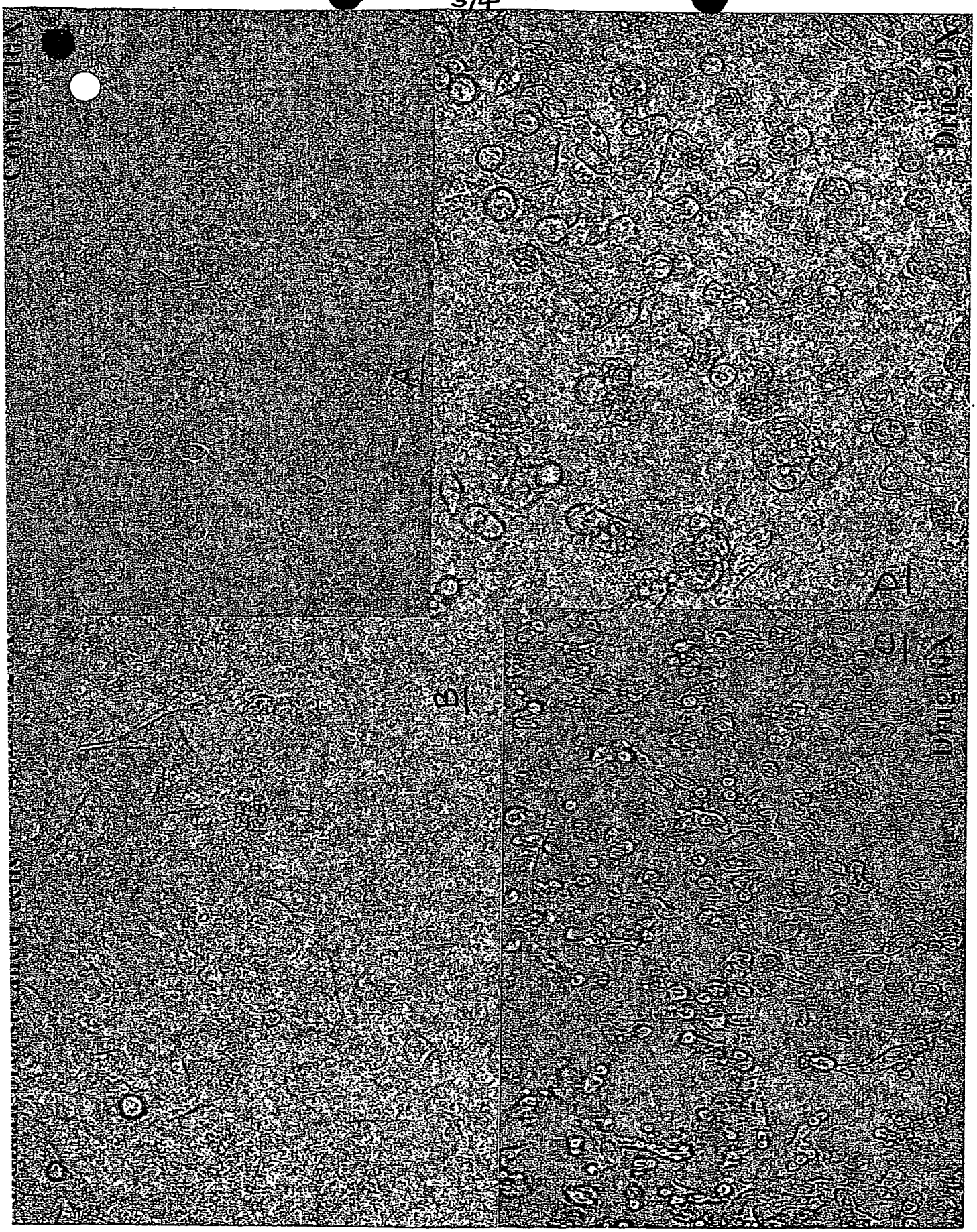

Fig3

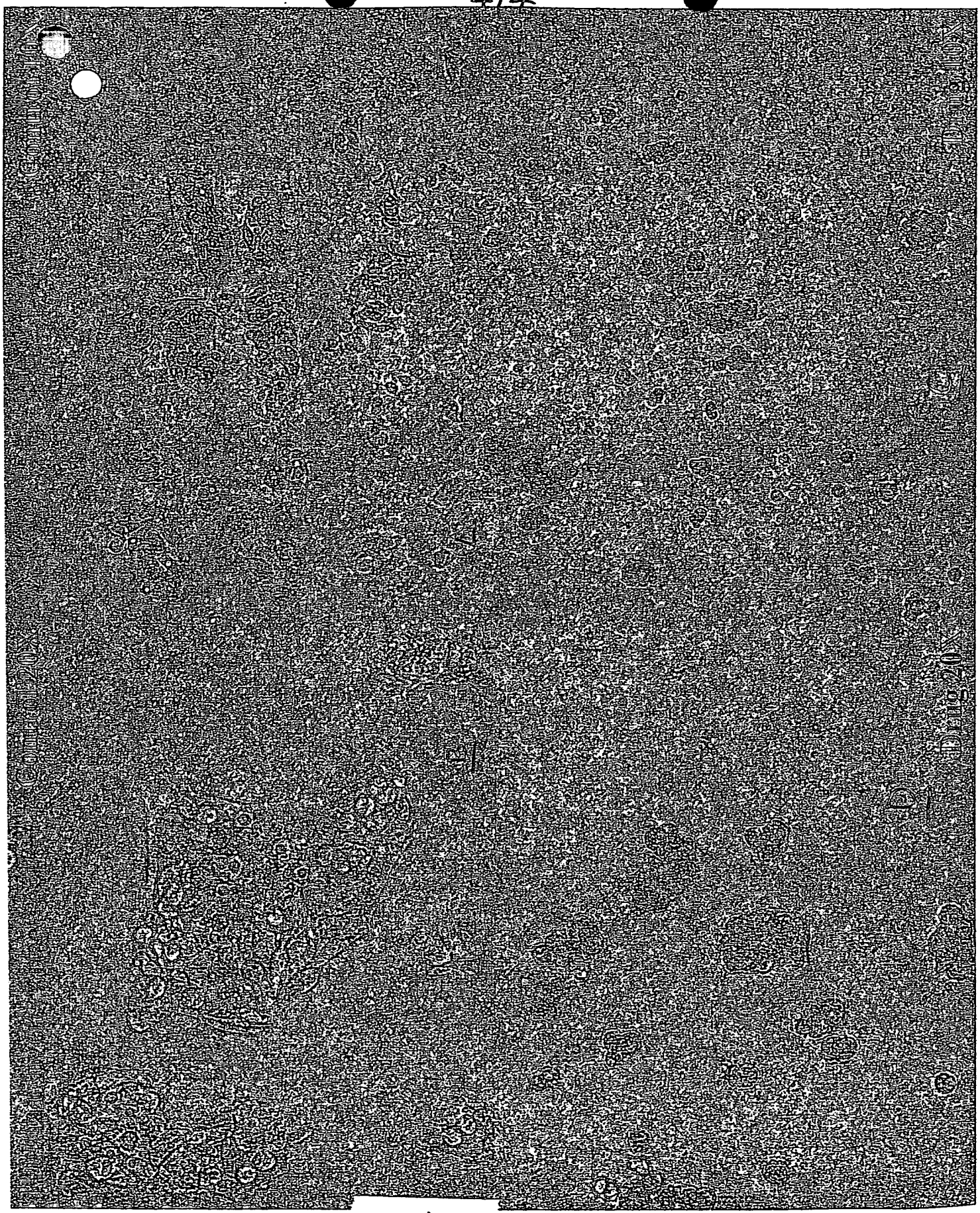

Fig 4